Leaf essential oils of *Juniperus* in central and southern Iran

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ABSTRACT

Leaf essential oils from *Juniperus* from southern Iran were analyzed and compared to oils of *J. excelsa, J. polycarpos, J. p.* var. *turcomanica* and *J. seravschanica*. The juniper oils from southern Iran were mainly in two groups: high cedrol (cf. *J. excelsa, J. polycarpos* and *J. seravschanica*) and low cedrol (cf. *J. p.* var. *turcomanica*). Complete analyses of the compositions are given.
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KEY WORDS: *Juniperus polycarpos* var. *polycarpos, J. seravschanica, J. p. var. turcomanica, J. excelsa,* Cupressaceae, Iran, terpenes, leaf essential oil.

The distributions of *J. excelsa* M.-Bieb., *J. polycarpos* K. Koch and *J. seravschanica* Kom. in Iran and the surrounding region are not well understood (Adams, 2011). Figure 1 summarizes our current understanding of these taxa's distributions. Adams and Hojjati (2012) investigated 10 populations of *Juniperus* in Iran using nrDNA, petN-psbM, trnD-trnT and trnS-trnG sequences (3705 bp). They found the northern populations, BL, Bj, Sh, were in a clade with *J. p.* var. *turcomanica* and L, H, and Q in a clade with *J. polycarpos* (Fig. 2). However, the southern populations displayed a mosiac pattern. One of

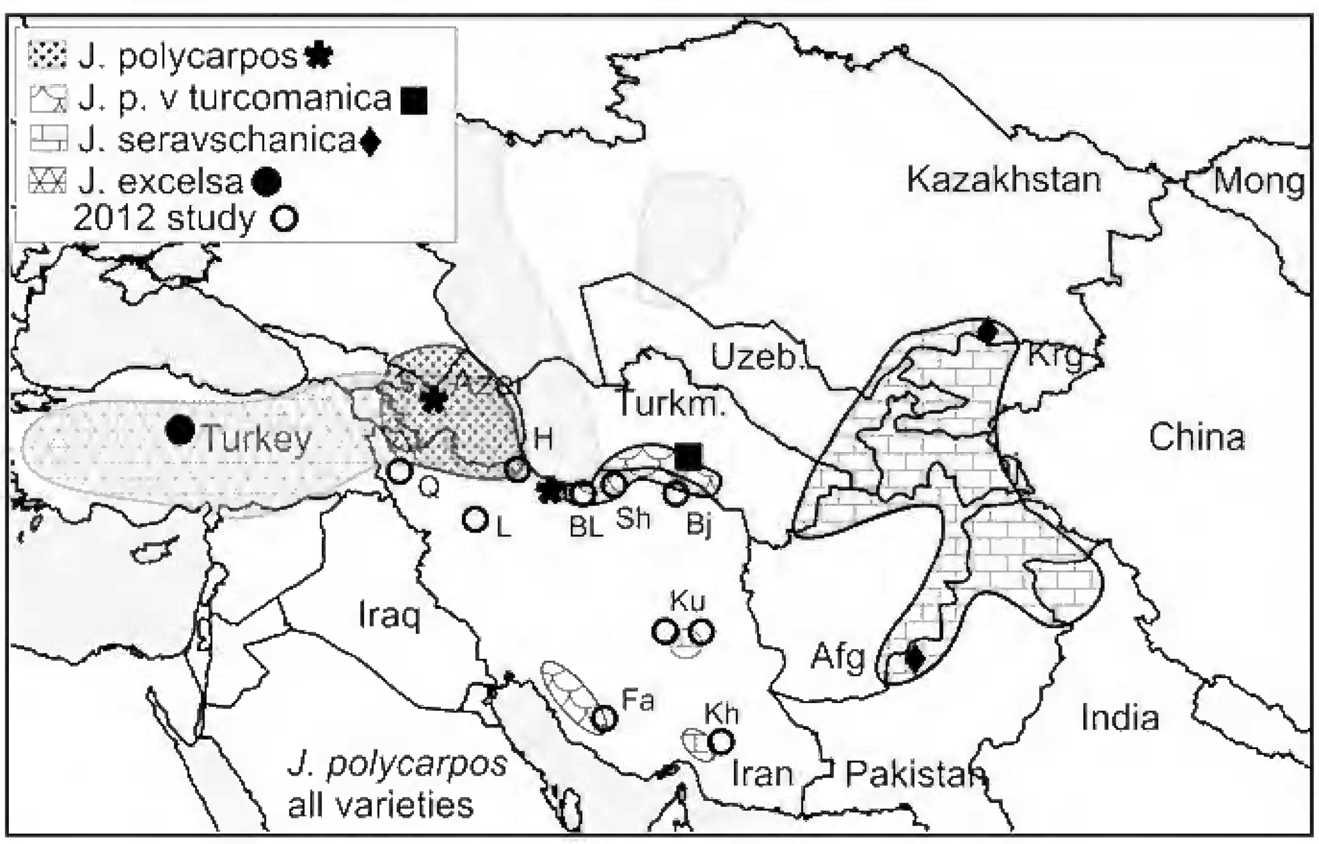

Figure 1. Distributions of *J. excelsa* (Greece not shown), *J. polycarpos* var. *polycarpos*, *J. p.* var. *seravschanica*, *J. p.* var. *turcomanica*. (adapted from Adams and Hojjati, 2012, symbols indicate the populations sampled for each taxon).

the samples from southern Kuhbanan population (Ku1) is loosely associated with the northern clade and a second sample (Ku2) is in a clade with Khabr (Kh1, Kh2) that is associated with *J. seravschanica*. Another perspective is shown in Figure 3, where the northern populations, L, H, Q, are clearly associated with *J. polycarpos*. Other northern populations BL, Bj, Sh, are loosely linked to var. *turcomanica* (Fig. 3). The Fasa samples (F1, F2) differ by only one SNP from BL in northern Iran. The samples from Khabr (Kh1, Kh2) are linked to *J. seravschanica* by 9 SNP differences (Fig. 3) and a sample from Kuhbanan (Ku2) is linked to the Khabr samples by 6 SNPs differences (Fig. 3). Clearly the junipers from southern Iran contain elements of *J. p.* var. *turcomanica* and *J. seravschanica*.

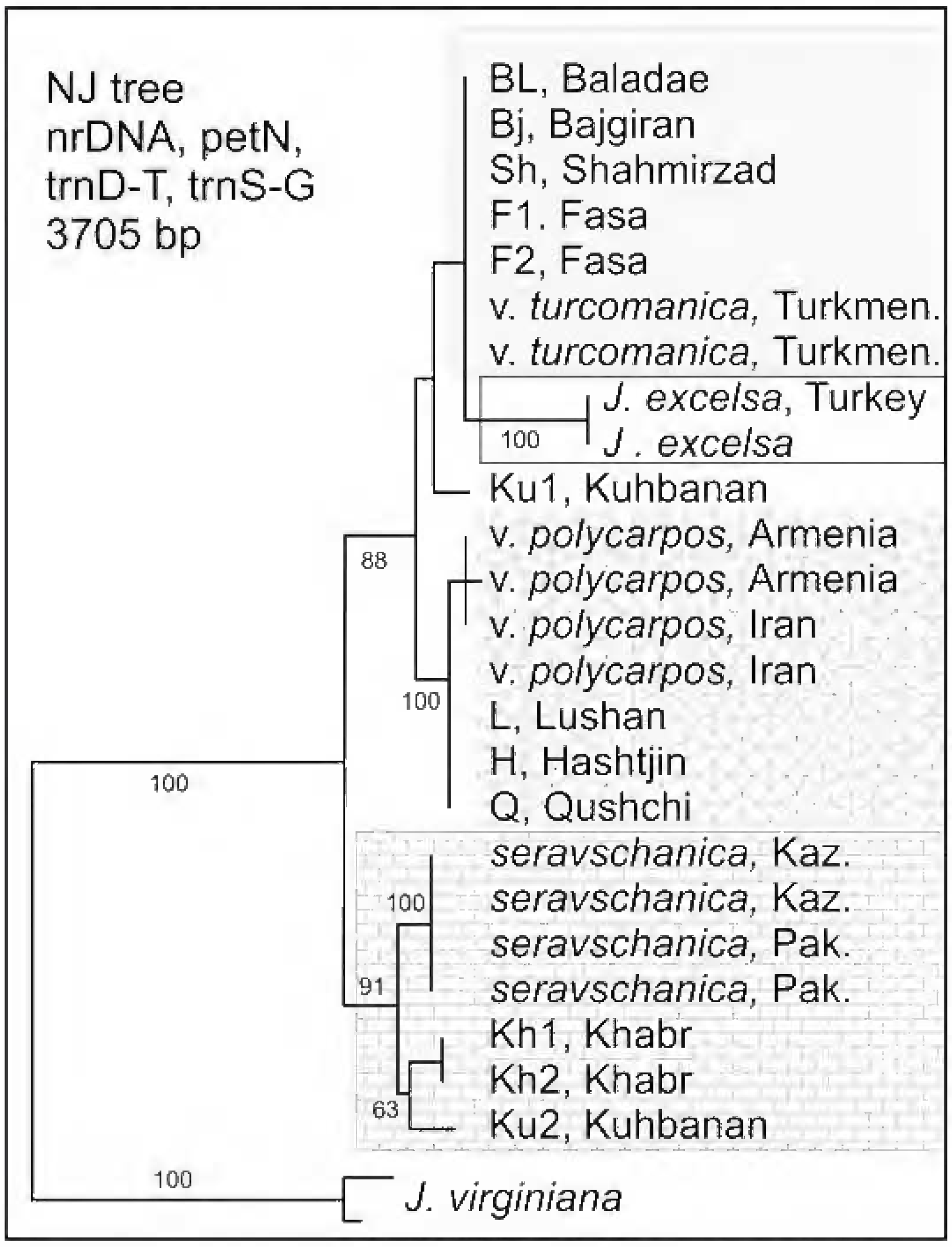

Figure 2. NJ tree of Iranian junipers (adapted from Adams and Hojjati, 2012)

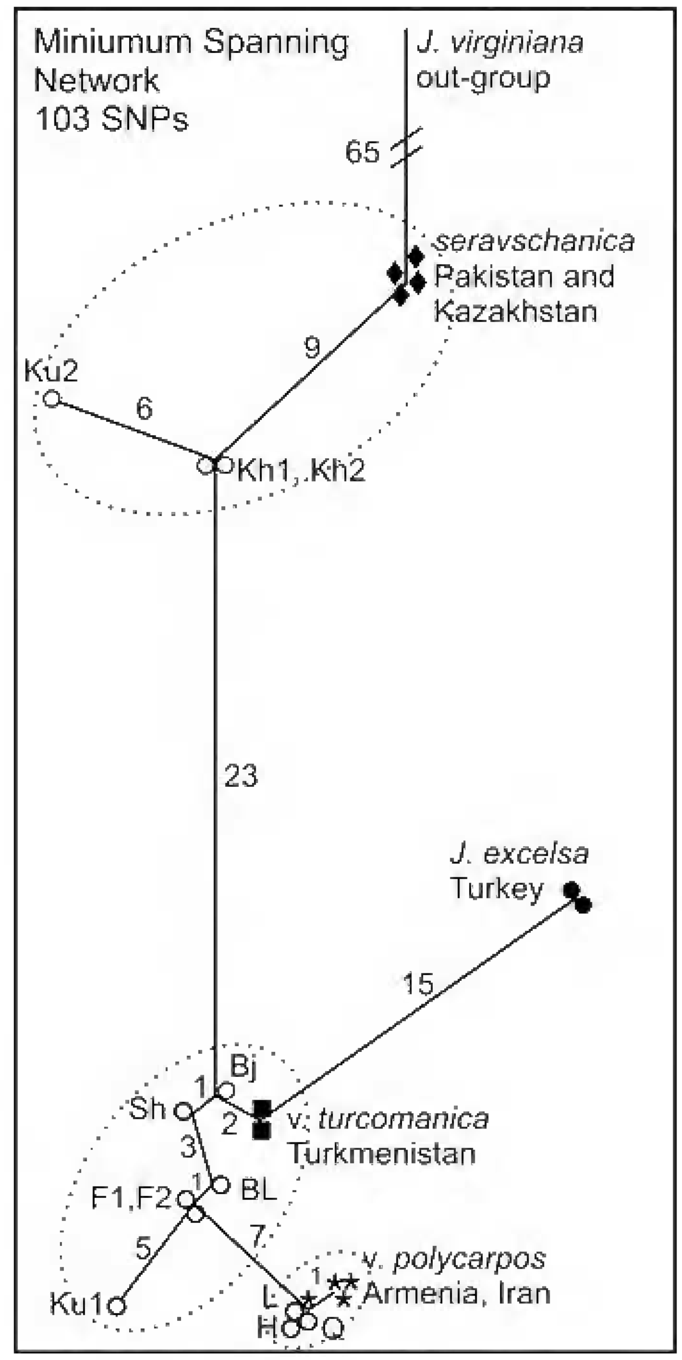

Figure 3. Minimum spanning network (adapted from Adams and Hojjati, 2012).

Adams and Shanjani (2011), using DNA sequence data, showed the juniper from the Elburz Mtns. to be typical *J. polycarpos* not *J. excelsa* as supposed. The composition of the leaf oils of *J. polycarpos* and *J. seravschanica* were previously reported by Adams (2001) and Adams et al. (2008). The leaf essential oils of *J. excelsa* have recently been reported (Adams et al. 2013).

The purpose of the present study is to investigate the leaf essential oils of the southern *Juniperus* of Iran.

MATERIALS AND METHODS

Plant materials (see Fig. 4):

Fasa (F1-F5), putative *J. polycarpos* var. *turcomanica*, 30 km past Fasa towards Neiriz, common on rocks. 29° 09' 51.1" N; 53° 44' 13.5" W, 1715 m, Oct. 2012, Prov. Fars, *F. Hojjati #1 to #5* (lab acc. *Adams 13754-13758*);

Kuhbanan (Ku)(K1-K9), putative *J. seravschanica* and *J. polycarpos* var. *turcomanica,* Kuh-e Bajgen, 55 km from Kuhbanan, Dolatabad, common on rocks. 31° 27' 12.8" N; 55° 52' 28.8" W, 2333 m, Oct. 2012, Kerman Prov., *F. Hojjati *1 to *9* (lab acc. *Adams 13759-13767*);

Khabr(KH)(B1-B5), putative *J. seravschanica,* Kuh-e Khabr. common on rocks. 28° 49' 06.7" N; 56° 21' 21.7" W, 2086 m, Oct. 2012, Prov. Kerman, *F. Hojjati -1 to -5* (lab acc. *Adams 12768-13772*);

Rabor (R)(R1-R5), putative *J. polycarpos* var. *turcomanica,* Gusichai village, 23 km past Rabor, between Rabor and Darbehest. 28° 49' 06.7" N; 56° 21' 21.7" W, 2086 m, Oct. 2012, Prov. Fasa, F. Hojjati .1 to .5 (lab acc. Adams 13773-13777).

Figure 4. Populations sampled in the present study.

Authentic, typical taxonomically identifiable reference taxa, were included from *J. excelsa,* n of Eskisehir, Turkey, *Adams 9433-9435*; *J. polycarpos* var. *polycarpos*, Lake Sevan, Armenia, *Adams 8761-8763*, *J. p.* var. *turcomanica*, Kopet Mtns., ca 140 km wnw of Ashgabat, Turkmenistan, 38° 25.12' N, 56 58.80' E, 1535m, *Adams 8757-8760*; *J. seravschanica*, Quetta, Pakistan, *Adams 8483-8485*, Dzhabagly, Kazakhstan. Voucher specimens are deposited at Baylor University (BAYLU).

Chemical analysis

Fresh, air dried leaves or herbarium specimens (20-100 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20ºC until analyzed. The extracted leaves were oven dried (100ºC, 48 h) for determination of oil yields.

The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. Terpenoids (as per cent total oil) were coded and compared among the species by the Gower metric (1971). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

The volatile leaf oils of the junipers of southern Iran were of three kinds and illustrated (Table 1) by samples Kuh6 (high cedrol), Kuh9 (low cedrol) and Kbr5 (low cedrol). All the oils were high in α-pinene (48.7 - 62.5%). Kuh6 (23.6% cedrol) was more like *J. excelsa*, (25.4), *J. polycarpos* (30.3) and *J. seravschanica* (13.8, 22.7) in having high amount of cedrol (Table 1). In contrast, Kuh9 (0.0 cedrol) and Kbr5 (0.1 cedrol) were like *J. p.* var. *turcomanica* (0.2% cedrol). However, several terpenes in southern junipers were in higher concentrations than found in *J. excelsa*, *J. polycarpos* or *J. seravschanica*: limonene, β-phellandrene, trans-verbenol, α-eudesmol, and β-eudesmol (Table 1).

To further examine the patterns, the similarity matrix was factored and yielded eigenroots accounting for 29.3, 11.1 and 7.1% of the variation among the 24 samples plus the 5 exemplar taxa. The eigenroots appeared asymptote after the third root. The large amount of variance extracted by the first eigenroot indicates that the major trend was the separation of 2 groups (high and low cedrol oils) on the first eigenvector (Fig. 5). The second eigenroot (11%) mostly separates the exemplar taxa from the 24 southern Iran samples (Fig. 5). The third eigenroot (7%) separates a sub-group of low cedrol trees from the bulk of the low cedrol trees (Fig. 5). A close examination of individuals reveals that both high and low cedrol oils are found in each of the four populations sampled.

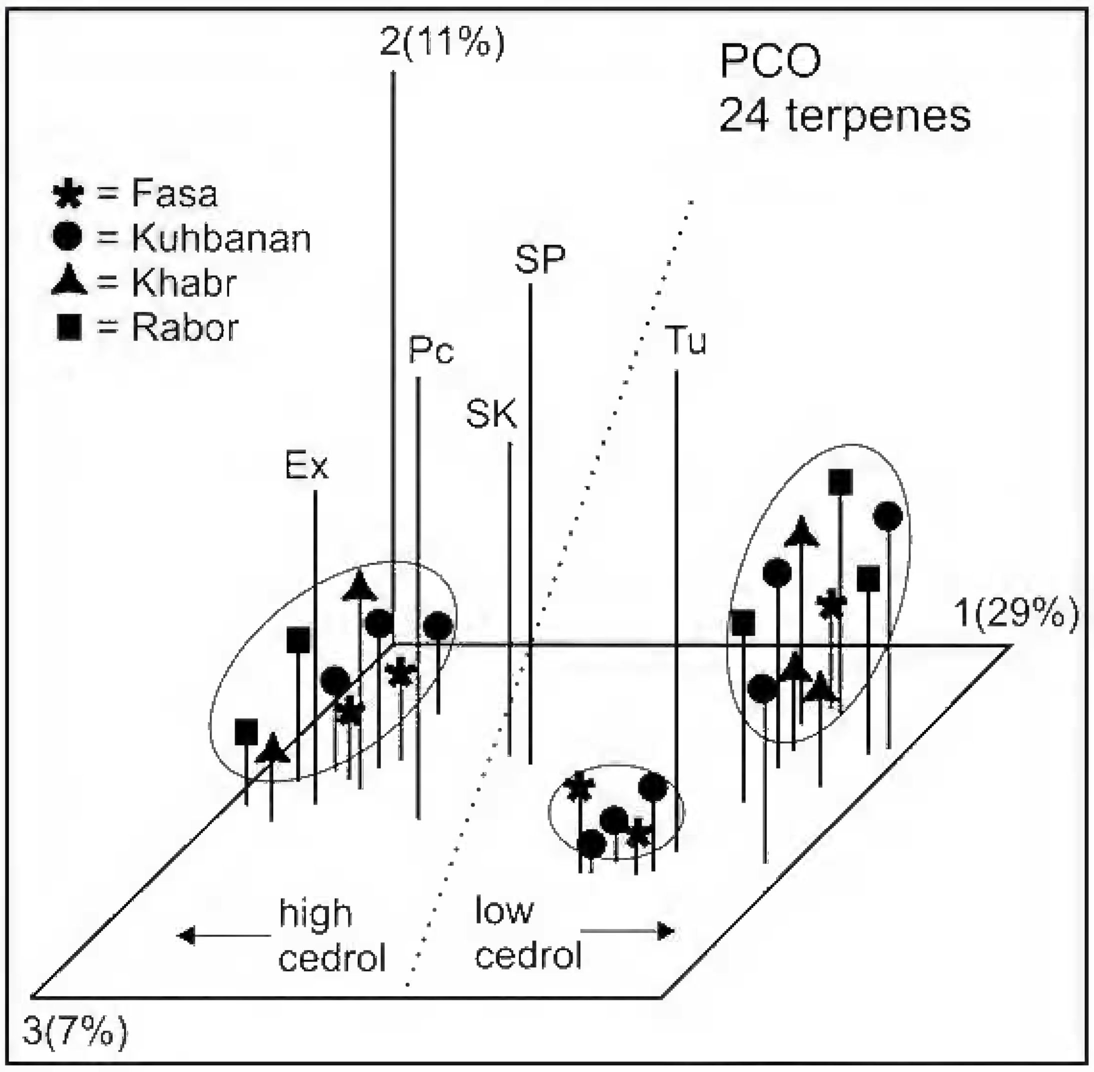

Figure 5. PCO of southern Iran *Juniperus* from 4 populations (see map, Figs. 1, 4), using 24 terpenes. The five exemplar oils are: Ex = *J. excelsa*, Turkey; Pc = *J. polycarpos*, Armenia; SK = *J. seravschanica*, Kazakhstan, SP = *J. seravschanica*, Pakistan; Tu = *J. p.* var. *turcomanica*, Kopet Mtns., Turkmenistan.

The low cedrol trees are loosely grouped with the *J. p.* var. *turcomanica* exemplar (Tu, Fig. 5). The sub-group of low cedrol oils (Fig. 5, fore-ground) is composed of 3 trees from Kuhbanan (filled circles, Fig. 5) and 2 trees from Fasa (stars, Fig. 5), with no samples from Khabr or Rabor. But the small sample numbers could have failed to include all populations in this sub-group.

The large amount of variation in the oils from each population is very likely due to hybridizing. The fact that many of samples displayed transgressive variation in several terpenoids, is suggestive of hybridization. Adams and Tsumura (2012) reviewed several papers on the inheritance of terpenes in conifers and found that terpenes seemed a little more likely to exceed the concentration (i.e., transgressive) of either parent (in a hybrid cross), than to be at intermediate levels. Hanover (1966) examined the monoterpene concentrations in 17 F_1 hybrids and their parents and found transgressive inheritance in 9/17 (α-pinene), 10/17 (β-pinene), 1/17 (δ-3-carene) and 6/17 (limonene) instances.

Cool et al. (1975), studying inheritance of terpenes in *Cupressus* hybrids, found 7/13 terpenes to be transgressive in the oils of hybrids. Adams and Stoehr (2013) analyzed the inheritance of terpenes in artificial hybrids of *Pseudotsuga menziesii* and var. *glauca*. They reported intermediate inheritance in 11/25 terpenes and transgressive inheritance in 14/15 terpenes. Adams and Tsumura (2012) analyzed artificial hybrids between cultivars of *Cryptomeria japonica* and reported intermediate inheritance for 7 terpenes and transgressive inheritance for 8 terpenes. Interestingly, the heartwood oils, cedrol, widdrol, cis-thujopsene, etc. were inherited as a group as a Mendelian dominate/ recessive fashion, with a second gene(s) as a modifier. This genetic system made the detection of hybrids very difficult; as hybrids' oils with heartwood components were nearly identical to one parent in the ordination. Removing the heartwood components from the data set aided the detection of hybrids, but a few hybrids still ordinated close to one parent.

It may be that DNA sequencing will aid in the understanding of variation in *Juniperus* in southern Iran (in progress). It seems likely that considerable field work will be needed resolve this situation.

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Table 1. Leaf essential oils for the multi-seeded junipers of Iran and adjacent areas. Exc - *J. excelsa*, 13193 (9433-35) Eskisehir; Poly - *J. polycarpos* var. *polycarpos*, 13194 (8761-63); SeraK - *J. seravschanica* - 13196 (8224-26); Kazakhstan, SeraP - *J. seravschanica*, 13195 (8483-85), Pakistan; Turco - *J. p.* var. *turcomanica* 13197 (8758-90); Kuh6, 13764, high cedrol, Kuhbanan; Kuh9, 13767, low cedrol, Kuhbanan, Kbr5, 13772, low cedrol, Khabr. Components in boldface were used in numerical calculations of similarities.

KI	Compound	Exc	Poly	SeraK	SeraP	Kuh6	Kuh9	Kbr5	Turco
926	tricyclene	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.1
931	α-thujene	-	t	0.3	0.4	t	t	-	t
939	**α-pinene**	**41.7**	**39.9**	**48.3**	**19.9**	**48.7**	**62.5**	**57.6**	**45.0**
953	α-fenchene	0.3	t	0.1	0.1	0.1	0.1	0.3	0.1
953	camphene	0.2	0.2	0.3	0.2	0.3	0.4	0.5	0.2
957	thuja-2,4(10)-diene	0.1	t	t	-	t	t	0.1	t
961	verbenene	t	t	t	t	t	t	t	t
976	sabinene	0.1	0.4	0.5	0.5	-	t	-	0.3
980	**β-pinene**	**0.7**	**0.5**	**1.2**	**0.9**	**1.4**	**1.1**	**1.0**	**0.8**
991	**myrcene**	**1.2**	**1.3**	**18.3**	**27.4**	**1.9**	**2.4**	**2.4**	**1.9**
1005	α-phellandrene	0.1	-	0.1	0.1	t	t	0.1	-
1011	**δ-3-carene**	**5.3**	**t**	**-**	**0.8**	**1.3**	**0.9**	**3.4**	**t**
1018	α-terpinene	0.1	t	0.1	0.1	t	t	t	t
1026	p-cymene	0.6	0.2	0.2	0.6	0.3	0.5	0.5	0.2
1031	**limonene**	**1.2**	**1.0**	**1.8**	**1.4**	**4.3**	**6.3**	**5.2**	**1.0**
1031	**β-phellandrene**	**0.9**	**0.4**	**1.3**	**1.3**	**2.8**	**4.2**	**3.5**	**0.8**
1050	(E)-β-ocimene	t	-	t	t	-	-	-	-
1062	γ-terpinene	0.5	0.3	0.7	1.0	0.3	0.5	0.4	0.4
1068	cis-sabinene hydrate	t	t	0.1	0.2	-	-	-	t
1088	**terpinolene**	**1.1**	**0.6**	**0.9**	**0.8**	**0.5**	**1.0**	**1.5**	**0.8**
1098	**linalool***	**-**	**0.1**	**0.3**	**0.8**	**3.8**	**2.6**	**0.8**	**t**
1112	endo-fenchol	t	-	-	-	-	0.2	0.1	-
1116	3-methyl butanoate, 3-methyl-3-butenyl-	t	-	-	-	t	-	-	0.1
1121	cis-p-menth-2-en-1-ol	0.1	-	t	-	-	-	-	-
1125	α-campholenal	0.5	0.3	t	0.1	0.1	0.2	0.2	0.2
1139	trans-pinocarveol	0.8	0.2	-	t	0.3	0.3	0.3	0.2
1140	cis-verbenol	0.2	t	-	-	t	t	t	t
1143	camphor	1.2	-	t	-	-	-	-	1.0
1143	**trans-verbenol**	**-**	**1.1**	**-**	**0.3**	**0.6**	**1.3**	**0.7**	**-**
1148	camphene hydrate	0.1	-	t	-	t	t	t	t
1159	p-mentha-1,5-dien-8-ol	0.1	t	-	t	-	-	-	0.1
1165	borneol	-	-	0.1	-	0.3	0.1	0.3	-
1172	cis-pinocamphone	0.2	t	-	-	t	t	0.1	-
1177	terpinen-4-ol	0.1	t	0.3	0.4	-	t	-	t
1179	naphthalene	0.1	0.2	t	-	-	-	-	0.6
1183	p-cymen-8-ol	0.1	t	t	-	t	-	t	t
1189	α-terpineol	t	t	0.1	t	0.2	0.2	t	t
1193	4Z-decenal	-	-	-	-	0.3	-	0.1	-
1204	verbenone	0.2	0.2	t	t	0.1	0.2	0.1	0.1
1217	trans-carveol	0.2	t	-	-	0.1	0.2	0.1	t
1241	isoamyl hexanoate	-	-	-	-	0.1	0.4	0.1	-
1243	hexyl 3-methyl butanoate	-	0.2	t	t	-	-	-	0.4
1252	piperitone	0.1	-	-	-	-	-	-	-
1257	4Z-decen-1-ol	-	-	-	0.1	0.6	t	0.3	-
1285	bornyl acetate	0.4	0.4	0.6	0.4	0.2	0.1	0.4	0.3
1286	linalool oxide acetate (pyranoid)	0.2	-	-	-	-	-	-	-
1290	trans-sabinyl acetate	-	-	-	0.2	-	-	-	-
1319	(2E,4E)-decadienal	2.4	-	0.1	0.3	-	-	-	t

KI	Compound	Exc	Poly	SeraK	SeraP	Kuh6	Kuh9	Kbr5	Turco
1320	149,91,77,164	-	-	-	-	-	0.3	-	-
1339	δ-elemene	-	t	t	t	-	t	-	0.1
1376	α-copaene	-	-	t	t	-	-	-	0.1
1382	hexyl n-hexanoate	-	0.2	-	0.2	0.3	0.5	0.6	0.1
1383	β-bourbonene	0.1	-	-	-	-	-	-	-
1389	β-elemene	-	-	t	t	-	t	0.2	0.2
1389	β-cubebene	0.1	0.1	t	-	-	-	-	t
1409	**α-cedrene**	**0.8**	**1.0**	**0.2**	**0.6**	**2.1**	**-**	**-**	**0.1**
1409	1,7-di-epi-β-cedrene	0.7	1.5	0.1	0.7	-	-	-	-
1418	**(E)-caryophyllene**	**-**	**0.8**	**0.1**	**0.3**	**-**	**0.3**	**0.4**	**0.6**
1418	β-cedrene	0.5	0.2	0.1	0.2	0.8	-	-	t
1429	cis-thujopsene	0.3	0.4	0.2	0.2	0.3	-	-	-
1434	γ-elemene	-	-	-	-	-	0.1	0.4	t
1446	cis-muurola-3,5-diene	0.2	-	t	-	-	-	-	t
1454	α-humulene	0.1	-	t	t	-	0.3	t	0.2
1458	(E)-β-farnesene	0.2	0.3	t	0.2	0.4	-	-	-
1461	cis-muurola-4(14),5-diene	-	0.1	t	t	0.1	-	-	0.2
1466	β-acoradiene	0.1	0.2	-	t	-	-	-	-
1473	trans-cadina-1(6),4-diene	0.2	-	t	-	-	-	-	-
1477	γ-muurolene	-	-	t	t	-	0.3	t	0.3
1480	**germacrene D**	**0.6**	**0.6**	**0.1**	**0.3**	**0.1**	**0.5**	**0.2**	**1.3**
1489	β-selinene	-	-	-	-	-	t	0.2	-
1491	trans-murrola-4(14),5-diene	0.2	-	t	0.2	-	-	-	0.2
1493	epi-cubebol	0.3	-	0.1	-	-	-	-	0.5
1494	bicyclogermacrene	-	0.3	-	t	-	-	-	-
1496	viridiflorene	-	-	-	-	-	0.3	0.3	-
1499	α-muurolene	0.1	-	0.3	0.2	-	-	-	0.7
1502	cuparene	0.1	-	-	t	-	-	-	-
1503	germacrene A	-	-	-	-	-	-	-	0.2
1509	β-bisabolene	0.1	t	-	t	0.1	-	-	-
1513	α-alaskene	0.2	0.1	-	0.3	0.1	-	-	-
1513	**γ-cadinene**	**-**	**1.0**	**0.4**	**0.7**	**-**	**1.6**	**-**	**1.7**
1513	cubebol	0.4	-	-	-	-	-	-	-
1521	β-sesquisphellandrene	-	-	-	-	0.2	-	-	-
1524	**δ-cadinene**	**0.5**	**0.8**	**1.1**	**0.8**	**-**	**1.6**	**t**	**2.8**
1532	E-γ-bisabolene	0.2	0.3	-	-	-	-	-	-
1532	γ-cuprenene	0.2	-	-	t	0.1	-	-	-
1533	sesquiterpene, <u>161</u>, <u>204</u>, 133,189	-	-	-	-	-	-	0.8	-
1538	α-cadinene	-	-	0.2	0.1	-	-	-	0.4
1545	selina-3,7(11)-diene	-	-	-	-	-	0.3	0.4	-
1549	**elemol**	**-**	**0.4**	**0.7**	**0.9**	**0.3**	**0.4**	**1.1**	**0.7**
1556	**germacrene B**	**-**	**1.6**	**0.5**	**0.7**	**0.3**	**1.4**	**6.3**	**2.8**
1574	**germacrene D-4-ol**	**-**	**1.5**	**1.2**	**2.9**	**-**	**2.0**	**-**	**8.9**
1587	**allo-cedrol**	**1.9**	**2.3**	**0.8**	**1.2**	**1.1**	**-**	**-**	**-**
1596	**cedrol**	**25.4**	**30.3**	**13.8**	**22.7**	**23.6**	**-**	**0.1**	**0.2**
1606	humulene epoxide II	t	-	-	-	-	-	-	-
1606	β-oplopenone	t	-	-	-	-	0.4	-	0.4
1627	1-epi-cubenol	0.5	-	-	-	-	-	-	-
1630	γ-eudesmol	-	0.4	-	-	-	-	0.4	-
1640	epi-α-cadinol	t	0.2	0.3	0.4	-	0.5	-	1.4
1640	epi-α-muurolol	t	0.3	0.4	0.4	-	0.5	-	1.4
1642	selina-3,11-dien-6-α-ol	-	-	-	-	-	-	0.2	-
1645	α-muurolol	t	t	0.1	0.1	-	t	-	0.5
1649	**β-eudesmol**	**-**	**t**	**0.2**	**0.2**	**0.3**	**0.3**	**1.0**	**0.2**
1652	**α-eudesmol**	**-**	**0.2**	**0.1**	**0.2**	**0.3**	**0.5**	**1.0**	**t**
1653	α-cadinol	t	0.5	0.9	1.0	-	0.5	-	3.6

KI	Compound	Exc	Poly	SeraK	SeraP	Kuh6	Kuh9	Kbr5	Turco
1661	sesquiterpene 85,57,41,238	1.0	-	-	-	-	-	-	t
1663	β-atlantone	0.6	-	-	-	-	-	-	-
1666	bulnesol	-	0.3	-	0.2	-	-	-	0.2
1666	(2E,4E)-decadienol	0.6	-	-	-	-	-	-	-
1688	**shyobunol**	**-**	**1.2**	**0.7**	**1.6**	**0.3**	**t**	**0.4**	**2.1**
1700	eudesm-7(11)-en-4-ol	-	-	-	-	-	-	0.3	-
1789	8-α-acetoxyelemol	-	-	-	t	-	-	-	0.1
1961	sandaracopimara-8(14),15-diene	t	-	-	-	-		-	-
1989	manoyl oxide	0.1	0.4	-	0.2	0.1	t	0.1	-
2054	abietatriene	0.1	t	-	t	-	-	t	t
2080	abietadiene	0.5	0.8	t	0.3	-	t	0.2	0.7
2147	abieta-8(14),13(15)-diene	-	-	-	-	-		-	t
2181	sandaracopimarinal	-	-	-	-	-		-	0.1
2288	**4-epi-abietal**	**0.2**	**1.5**	**0.1**	**1.2**	**0.1**	**0.2**	**0.5**	**1.9**